REMARKS

The fee for the three month extension of time should be charged to Deposit Account No. 02-1818. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 02-1818. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-3, 5-8, 14, 15, 17-19, 22-33, 43, and 44 are pending in the application. Claims 1 and 27 are amended to render it clear that each entire process is performed in a high throughput format. Basis can be found throughout the specification, which repeatedly (including in the title) describes the process as high throughput. The application defines highthroughput screening as processes that test a large number of samples at once.

THE CLAIMED SUBJECT MATTER

Before addressing the rejections under 35 U.S.C. §103, the subject matter of the claims is reiterated. The method is a high throughput method for modifying a target polypeptide to have a change in a **predetermined** property or activity (*i.e.*, is a directed protein evolution method). The method produces Lead proteins that have a predetermined evolved property or activity The method involves **first replacing <u>every</u> amino acid along the <u>full-length</u> of the protein with a pre-selected amino** acid and testing **<u>each and every</u>** resulting protein for the property or an activity to identify any that have a change in the activity or property. This is performed in a highthroughput format, so that, while each amino acid is individually replaced along the full-length of the protein, this is effected, a plurality of proteins are produced, such that all of the positions along the full-length of the protein are individually modified and screened. This changing and testing and is effected along the full length of the protein and is done individually in a high throughput format, so that each protein is separately produced and separately screened, but each step is performed on all sets at once (*i.e,* in a high throughput format) so that every residue along the full-length of the protein is tested to identify hits. As a result there is no bias created by screening mixtures of molecules or creating mixtures so that all hits are identified without any bias that occurs when mixtures of mutations are added or multiple mutations are included or mixtures are screened..

In the second part of the method as claimed, each hit locus is then individually replaced with every other amino acid and again individually screened to identify LEADS; LEADS differ in the predetermined activity or property from the hit, typically exhibiting a

desired activity or property. Again bias in production, expression and/or screening, is avoided because all modifications at hit positions will be individually expressed and screened. In addition, because each modified protein is individually modified at a single separate locus, the identity of the protein at each locus is known prior to screening, so that upon identification of a hit, no sequencing or other identification is needed. All possible changes in the protein are tested for their effect on the predetermined activity; not just a subset. As a result, every position in the protein that affects the activity/property (part 1 of the method) and every amino acid change the affects the activity/property as predetermined is identified. As stated above and in the application, the method, thus, is unbiased, since everything is done individually and all residues are tested.

As shown in the application and discussed previously, the application exemplifies practice of the method with AAV Rep protein-encoding nucleic acid to identify mutations that result in production of higher titer AAV. While AAV viruses with mutated Rep proteins previous have been produced, **none in the prior art were ever identified that** result in higher titer. Application of the instantly claimed method, in which every position along the length of the nucleic acid molecule was replaced and tested to identify hits, and then each hit was replaced with every other amino acid, however, resulted in identification of Rep protein mutations that result in higher titer. At least 6 mutations that result in higher titer of AAV virus were identified, thereby demonstrating the power of the method. Because the method essentially tests every residue to identify those that alter a particular activity or property and the screens every replacing amino acid at those positions and does so individually, it ferrets out valuable mutations that are missed by prior art methods that somehow limit or bias the screening, such as by expressing or screening mixtures or failing to test all loci in the protein. Since it is performed in a high throughput format it is feasible to produce and screen every molecule.

During the extensive prosecution of this application, no art, singly or in combination, that teaches the method as claimed has been identified; all rejections have been obviated. The Office, as in this case, recasts rejections. As discussed below, the currently cited art, new applied, to the extent pertinent, only are pertinent to modification of domains of a protein. Neither reference teaches or suggests a directed evolution method, and neither suggests the steps of the instantly claims methods. Wells *et al.* in its investigation of active domains, does not provide a directed evolution method; Wells *et al.* investigates

structure/function. Wells *et al.* does not provide any method for identifying all residues that contribute to a particular predetermined activity, nor does it teach a method for doing so. After identifying a putative domain, Wells *et al.* literally separately produced and screened modified proteins to assess the effect of the residue on the activity of the domain, in order to identify residues important for activity in the domain, not to evolve a protein to have a particular predetermined activity. Pederson *et al.* teaches a method of clonally separating cells, which is not even applicable to the method of Wells *et al.* If Pederson e is cited for the proposition that Wells *et al.*, could have made the its mutants in microtiter plates, practicing the Wells *et al.* method in microtiter plates does not result in the instantly claimed methods.

As emphasized below, none of the references, singly or in any combination, teaches changing each residue in protein, one-by-one, and testing them one-by-one so that the effect of every residue is assessed. Wells *et al.*, which in fact is a method for identifying active domains, only changes residues in a particular domain after identifying such domain. Wells *et al.* is not directed to a method of protein evolution and does not teach or suggest modifying every residue one-by-one, nor doing so in a high throughput format. Wells *et al.* happens in its experiments, having identified a domain, changed residues in the domain, but did not teach or suggest any high throughput method nor any directed evolution method. The secondary references do not cure this deficiency.

In an effort to advance subject matter to allowance, while none of the art, singly or in combination teaches or suggests modifying the full-length of the protein as claimed, the claims are amended to render it clear the process is conducted in a high throughput format.

THE REJECTIONS OF CLAIMS 7, 24-29, 32, 33 UNDER 35 U.S.C. §103(a)

Several grounds of rejection are set forth under 35 U.S.C. §103(a) The particular grounds of rejection are discussed in turn below. As discussed below, neither Wells *et al.*, and Pederson *et al.*, singly or in combination, nor any reference of record, nor any combination of references, teaches or suggests steps (a) -(d) nor steps (a)- (g) of claim 1 and dependents nor steps (a)-(f) of claim 27 and dependents as outlined above and as recited in each of the independent claims. None of the references teaches or suggests changing one amino acid at a time along the full length of the protein such that each protein with a single change is individually produced, expressed and screened so that **all** hits along the full-length or domain are identified. None suggests separately introducing each nucleic acid molecules cells of one locus of an array to produce an addressable array in which the identity of the

encoded protein is known *a priori*. None suggests then, after identifying hits by screening every amino acid position along the full length of the protein, individually modifying, expressing and screening each hit with every amino acid (remaining) to identify the particular changes that lead to the desired change in activity or property (i.e. the evolved property).

In addition, as exemplified in the application, the methods of the instant application are very powerful, permitting preparation of modified polypeptides that have a predetermined property or activity. In the working example in the application, the overlapping Rep protein-encoding gene(s) of AAV are changed one codon at a time along the full-length of the open reading frames encoding the Rep proteins to identify loci (hits) whose modification alters AAV titer. Each hit is then changed to every other amino acid to identify modified proteins that result in higher titer. The methods identifies at least 6 such proteins in each serotype. Heretofore, no Rep mutants had ever been identified that result in increased titer. Hence the method permits evolution of protein (*i.e.*, Rep proteins) to exhibit a predetermined property (*i.e.*, higher titer).

As discussed below, Wells *et al.* does not teach or suggest modifying a protein along its full-length, as Wells *et al.* is directed to a method for analysis of the structure and function of polypeptides by identifying active domains which influence the activity of the polypeptide with a target substance. Wells *et al.*, **does not teach a method for directed evolution**; Wells *et al.*, teaches methods for analyzing structure/function relationships of polypeptides by identifying active domains. Wells *et al.* is not modifying a protein to produce a modified protein that has a predetermined activity of property. The instantly claimed method, in which the protein is modified along its full length does not involve identifying active domains It is a high throughput method for systematically identifying all residues in a protein that affect a particular property/activity and then modifying such residues to produce a protein that has a predetermined activity/property. Wells *et al.* does not teach a method for evolving an activity/property of a protein; does not teach a high throughput method (only a limited number of molecules are made and tested by Wells); does not teach any of steps (a)-(d) of any pending claims, nor steps (a)-(g) (claim 1) or (a)-(f) (claim 27) of the pending claims. Pederson is merely directed methods for creating and screening spatial arrays that contain a single cell at each locus. Pederson is of no relevance to the instantly claimed methods; the instant claims do not require any method for spatially arraying a single clone. In the

instantly claimed methods, since each nucleic acid molecule is individually produced, and separately arrayed, there is no need to clonally select. Further, the method of Pederson does not result in an addressable array in which the identity of each clone at each locus is known *a priori*. Picking a clone from among a mixture and arraying each as in Pederson, does not result in an array in which the identity of each protein at each locus is known *a priori*. Wells *et al.* does not even produce clones, but produces a series of mutant polypeptides, each of which is individually produced. There would be no reason to spatially array a single clone. Clearly combining Wells *et al.* with Pederson *et al.* does not resulting in the instantly claimed methods as neither reference teaches a high throughput method, any of teach any of steps (a)-(d) of any pending claims, nor steps (a)-(g) (claim 1) or (a)-(f) (claim 27).
Thus, as discussed below, the combination of teachings of the references does not teach or suggest or result in the instantly claimed methods.

RELEVANT LAW

To establish prima facie obviousness under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness was not altered by the recent Supreme Court holding in KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82 USPQ2d 1385 (2007). In KSR, the Supreme Court stated that "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). See also KSR, 127 S.Ct. at 1734, 82 USPQ2d at 1391 ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.") The Court in Graham noted that evidence of secondary considerations, such as commercial success, long felt but unsolved needs, failure of others, etc., "might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented." 383 U.S. at

18, 148 USPQ at 467. Furthermore, the Court in KSR took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit the analysis supporting a rejection under 35 U.S.C. 103, stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. Id. at 1740-41, 82 USPQ2d at 1396 (citing In re Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)).

While the KSR Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. KSR, 127 S. Ct. at 1731. The court stated in dicta that, where there is a "market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try **might** show that it was obvious under § 103."

In a post-KSR decision, PharmaStem Therapeutics. Inc. v. ViaCell. Inc., 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would not be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

Furthermore, KSR has not overruled existing case. See In re Papesch, (315 F.2d 381, 137 USPQ 43 (CCPA 1963)), In re Dillon, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991), and In re Deuel (51 F.3d 1552, 1558-59, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995)). "In cases involving new compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007).

The mere fact that prior art may be modified to produce what is claimed does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

As always, unexpected properties must always be considered in the determination of obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesch*, 315 F.2d 381, 391, 137 USPQ 43, 51 (CCPA 1963)

The disclosure of the applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. In re Laskowski, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

In the instant case, the combination of teachings of the references does not teach or suggest all elements of the methods as claimed, the proposed combination would necessarily change the operation of the prior art method, and none of the cited references nor any combination thereof teaches or suggests changes in the cited art that would result in the instantly claimed methods. To combine the references to produce to claimed methods would require use of the instant application as a guide, involving the impermissible use of hindsight.

Rejection of claims

Claims 1-3, 5-8, 14, 15, 17-19, 22, 23, 27, 43 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner *et al.* (US 5,223,409) in view of Wells *et al.* (US 6,013,478) and in view of Pedersen *et al.* (W001/32844)].

First it does not appear that the rejection relies on Ladner *et al.*, but in fact is new ground of rejection based on Wells *et al.* in view of Pederson *et al.* since the Examiner agreed

that Ladner *et al.* is not relevant and states the rejection was withdrawn. Hence, the discussion below assumes that the rejection inadvertently recited Ladner *et al.*.

The Examiner states that:

> The instant claims are drawn to a method of producing a plurality of separate sets of nucleic acid molecules where each set of nucleic acid molecules differ from another set of nucleic acid molecules by one codon and the protein encoded on each set differ by one amino acid from the protein encoded on another set. The nucleic acid molecules are then introduced into host cells and an addressable array of these host cells is created where the location of each host cell and nucleic acid molecule is known. The host cells express the proteins and the proteins are individually screened to identify which proteins have a predetermined property that is a chemical, physical, or biological property or activity.

Second Applicant notes that this is an incorrect description of the claims; essential elements are omitted. For example, he instantly claimed method is a "A high throughput process for the identification of a protein that differs in a predetermined property or activity from a target protein; it is not a method for "producing a plurality of separate sets of nucleic acid molecules where each set of nucleic acid molecules differ from another set of nucleic acid molecules by one codon and the protein encoded on each set differ by one amino acid from the protein encoded on another set . . .." It is a directed protein evolution method. Claim 1 also requires, for example, producing a **plurality** of separate sets of nucleic acid molecules that encode modified forms of a target protein, where a **sufficient number of sets** of nucleic acid molecules are produced so that each encoded amino acid residue in the encoded protein is replaced with a pre-selected amino acid along the full-length of the encoded protein **so that all positions along the full-length of the protein are individually modified for screening,** and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein. Furthermore, claim 1 includes sets e-g and claim 27 includes sets d-f, which describe part 2 of the method in which each hit position is replaced with every remaining amino acid and individually screened to identify those having the predetermined activity. These steps are completely omitted from the Examiner's description of the claims.

As amended claim 1 explicitly recites that the method is performed in a high throughput format, such that all of the resulting encoded proteins, while individually produce and screened, are all produced at the same time and screened at the same time. Neither Wells *et al.* nor Pederson *et al.* teaches or suggests a these elements.

The Examiner states that:

> Wells *et al.* teaches a method of producing a plurality of separate sets of nucleic acid molecules that encode modified forms of a target protein (page 6, paragraph 45-65), where a residue substituted DNA sequence encodes a residue substituted polypeptide where each polypeptide contains a single substitution at a different amino acid within each segment of a protein (Wells describes proteins as being comprised of segments) (i.e. where each protein encoded by a nucleic acid molecule set differs from the other proteins by only one amino acid and where the amino acid residues are replaced along the full-length) (column 6, lines 44-65, column 15, line 51-column 16, line 11); expressing the encoded proteins (column 13, lines 3653); and screening the encoded proteins for a predetermined property that differs from the target protein by at least a 100% change (i.e. two-fold increase or decrease in Kd as compared to the parent protein) (column 17, lines 29-43); identifying each mutated protein as a hit and a designated hit position (i.e. active amino acid residues) (column 17, lines 29-43). Furthermore, Wells *et al.* teach modifying the nucleic acid molecules that encode the hits to produce more nucleic acid molecules by replacing codons in a hit position to produce nucleic acid molecules that differ by at least one codon (i.e., proteins that differ by one amino acid residue) (column 15, lines 50-11; column 17, lines 44-60); introducing the nucleic acid molecules into cells and screening the proteins for a predetermined activity (column 13, lines 36-53; column 17, lines 29-43; column 18, lines 25-43).

Second, as discussed previously an in more detail below, Wells *et al.* teaches identification of active domains, and replacement of amino acids in the domain, **not along the full length**, and **not** in a high throughput format. Wells *et al.* does not teach or suggest any high throughput format. Wells does not teach or suggest replacing hit positions with every amino acid and testing each such resulting protein (part 2 of the instantly claimed method).

The Examiner continues by stating that Pedersen *et al.* teaches a method of creating an array of host cells in a spatial array, which, if Wells put it cells in such a physical array would become addressable. **Third, as discussed in more detail below,** Wells is not performing a high throughput method, but individually produced a relatively small number of modified proteins to scan the identified domain. There is no suggestion in Wells *et al.* for performing its experiments in a high throughput format. Pederson *et al.* provides no reason to do so. Even if Wells put its cells into a physical array that does not result in a high throughput process, nor does it result in the instantly claimed method (as discussed below and previously).

The Examiner concludes that:

> It would have been obvious to one of ordinary skill in the art to use the addressable arrays of Pedersen *et al.* with the method of Wells *et al.* Wells *et al.* teaches introducing nucleic acids into host cells for the purposes of producing modified proteins. In order to screen the different types of proteins, one of ordinary skill in the art would have been motivated to use a high-throughput

method as taught by Pederson *et al.* to gain the advantage of a practical and reliable method for the identification of novel substances with new properties from a large number of molecules (page 37, lines 19- 24). Thus one of ordinary skill in the art would have been motivated to combine the method so Wells *et al.* and Pedersen *et al.*

This rejection respectfully is traversed.

The rejected claims

The claims are discussed above. Claims 1 and 27 are the only independent claims/ Each recites all steps to produce leads. Claim 1 recites:

A **high throughput** process for the identification of a protein that differs in a predetermined property or activity from a target protein, comprising:

(a) producing a **plurality of separate sets of nucleic** acid molecules that encode modified forms of a target protein, wherein:

the nucleic acid molecules in each set are produced by changing one codon in the target protein to a pre-selected codon, whereby the nucleic acid molecules in each set encode proteins that differ from the encoded proteins in another set by one amino acid;

a sufficient number of sets of nucleic acid molecules are produced so that each encoded amino acid residue in the encoded protein is replaced with a pre-selected amino acid along the full-length of the encoded protein so that all positions along the full-length of the protein are individually modified for screening, and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein; and

all nucleic acid molecules in a set encode the same modified protein;

(b) individually introducing each set of nucleic acid molecules into host cells to produce an addressable array of host cells, whereby the identity of each set of nucleic acid molecules in host cells of each locus in the array is known, wherein the cells of each locus of the addressable array contain the same modified nucleic acid molecules;

(c) expressing the encoded proteins, whereby a **plurality of separate sets of proteins** encoded by the nucleic acid molecules are produced **and all positions along the full-length of the protein are individually modified**, wherein:

all of the encoded proteins in each set have the same modification; and

the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid; and

(d) individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target , wherein:

each identified protein is designated a hit;

each hit contains a mutation designated a hit position; and

the predetermined property or activity is selected from among a chemical, a physical and a biological property or an activity of the target protein;

(e) modifying the nucleic acid molecules that encode the hits to produce sets of nucleic acid molecules that encode modified hits, wherein:

the modified hits are produced by systematically and individually replacing each codon that is a hit position with a codon encoding another amino acid to produce

nucleic acid molecules each differing by at least one codon and encoding modified hits; each set of nucleic acid molecules is individually designed and synthesized., whereby:

a **sufficient number of sets are produced to produce encoded proteins in which every hit is separately replaced with all other amino acids,** and the encoded protein in each set differs from the encoded protein each other set and the target protein by one amino acid ;

the identity of each set of nucleic acid molecules in host cells of each locus in the array is known and wherein the cells of each locus of the addressable array contain the same modified nucleic acid molecules;

(f) separately introducing each set of nucleic molecules that encodes the modified hits into cells to produce an addressable array of cells, whereby the identity of each encoded protein at each locus in the array is known, and expressing the protein encoded by the introduced nucleic acid molecules; and

(g) **screening all cells that** contain the expressed protein by individually screening each set of cells that contains the nucleic acid molecules that encode the modified hits **to identify one or more nucleic acid molecules that encode(s) a protein or the coded protein that has/have the predetermined property or activity that differs from the target protein and has properties that differ from the original hits,** wherein each such protein is designated a lead,

wherein each and all of steps (a)-(g) are performed in an automated high throughput format whereby each molecule is individually designed, produced, screened and tested in the high throughput format .

Claim 27 recites:

A **high throughput** process for the identification of a protein that differs in a predetermined property from a target protein, comprising:

(a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein:

each encoded modified protein in a set differs from the encoded proteins in each other set and from the target protein by one amino acid;

a sufficient number of sets of nucleic acid molecules are produced so that each encoded amino acid residue in the encoded protein is replaced with a pre-selected amino acid along the full-length of the encoded protein so that all positions along the full-length of the protein are individually modified for screening, and each nucleic acid molecule encodes a protein that differs by one amino acid from the target protein; and

the members of each set encode the same modified protein;

(b) individually, but at the same time, introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein:

the host cells are organized in an addressable array, whereby the identity of each nucleic acid molecule at each locus in the array is known;

each set of nucleic acid molecules is introduced into host cells at a different locus of the array, whereby the identity of each set of nucleic acid molecules in host cells at each locus of the array is known, wherein:

the cells of each locus of the addressable array contain the same modified nucleic acid molecules;

all encoded proteins in each set contain the same modification; and

the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid; and

(c) individually, but at the same time, screening the sets of encoded proteins to identify one or more proteins, designated hits, that have a predetermined property that differs from the target protein is/are identified, wherein:

each identified protein is designated a hit;

each hit contains a mutation designated a hit position; and

the predetermined property is selected from among a chemical, a physical and a biological property of the target protein;

the nucleic acid molecules comprise viral vectors; and the cells are eukaryotic cells that are transduced with the vectors;

(d) modifying the nucleic acid molecules that encode the hits to produce a set of nucleic acid molecules that encode modified hits, wherein each nucleic acid is in a viral vector;

(e) introducing each set of nucleic acids that encode the modified hits into cells; and

(f) individually, but at the same time, screening the sets of cells that contain the nucleic acid molecules that encode the modified hits to identify one or more cells that encodes a protein that has a predetermined property or activity that differs from the target protein and has properties that differ from the original hits, wherein each such protein is designated a lead, **wherein each and all of steps (a)-(f) are performed in an automated highthroughput format whereby each molecule is individually designed, produced, screened and tested in the high throughput format.**

As discussed above, all of the claims are directed to **high throughput** methods of directed evolution in which a target protein is modified one amino acid at a time along its full length, and each and every modified protein is separately expressed and screened separately from the other modified proteins. One amino acid change is introduced per protein, not one or more changes, but one change. Mixtures of different proteins are not produced, nor are mixtures screened. In addition, because one change is made at a time and the nucleic acid molecules encoding each protein are introduced host cells at different loci in an array the identity of the nucleic acid at each locus in the array, and, thus the protein, is known. The identified hits are then further modified by replacing each hit with other amino acids (claims 1 and 27), including all remaining amino acids (claim 1) and each such further modified hit is individually produced and screened.

As described in the application the method does not rely on any methods in which there is differential modification or expression of a particular modified protein nor, for the claimed embodiments, are particular regions scanned. For example, at page 24, lines 6-24, the specification states:

The whole process of the identification of the active site(s) on the full length protein sequence requires the following sub-steps:

a. Generation of a mutant library (on the gene to be evolved) in which each individual mutant contains a single mutation located at a different amino acid position and that includes a systematic replacement of the native amino acid by Ala or any other amino acid (always the same throughout the entire protein sequence);

b. phenotypic characterization of the individual mutants, one-by-one and assessment of mutant protein activity;

c. identification of those mutants that display an alteration, typically a decrease, in the selected protein activity, thus, indicating that amino acids directly involved in the active site(s) have been hit. The aa positions whose aa-scan mutations display an alteration, typically a loss or decrease, in activity are named HITS.

The identification of the active site(s) (HITS) is thus, by this method, made in a completely unbiased manner. There are no assumptions about the specific structure of the protein in question nor any knowledge or assumptions about the active site(s). The results of the amino acid scan identify such sites.

Similarly, after identification of hits, each and every hit is replaced with every amino acid and each such-modified protein is separately expressed and screened. As a result no bias is introduced by virtue of modification, expression or screening.

Hence, there is no bias introduced into the process. Amino acids are individually replaced and each variant is tested separately. There is no effect of differential expression in culture in which conditions could favor one variant over another nor effects of differential mutation, in which one locus might be more amenable to modification. Further, as exemplified, the method is quite powerful, permitting evolution, for example, of AAV Rep proteins to produce modified AAV that has increased titer. Further the method identified all modifications in the protein that result in increased titer. While modifications in the viral Rep genes was known, there had been no mutations identified that result increased titer. The instantly claimed unbiased systematic rational method permitted mutations that result in increased titer to be identified.

Teachings of each cited reference and differences from the claimed methods

Wells *et al.*

Wells *et al.* teaches a **method for identifying active domains** in a protein, and amino acid mutation of residues **in the active domain** to generate variant proteins with altered activity that conforms to the activity or a particular target protein. Wells *et al.* is concerned with domains, not the full-length polypeptide. In Wells *et al.*, **the active domains are identified** by substitution of regions of the protein with one or more analogs of the polypeptide of interest, which analogs exhibit a different activity from the polypeptide. These segment-substituted polypeptides are produced and contacted with a target for the

parent polypeptide to determine if the substituted region alters the interaction with the target. The active domain of the polypeptide is identified to be those segment substituted regions that confer a change in activity of the segment substituted polypeptide, such as a change in the Kd, relative to a parent polypeptide. Thus in step 1 of the method of Wells *et al.*, segments of the protein are replaced to essentially identify hits, *i.e.*, the regions that confer a change in activity. In the part 2 of method of Wells, once the active domain is identified, a scanning amino acid is substituted for an amino acid in an active domain of the parent polypeptide, **not along the full-length of the polypeptide**. A plurality of residue-substituted polypeptides are prepared, which each contain a single substitution in a different amino acid residue of the active domain with the same scanning amino acid. A plurality of polypeptides containing substituted residues are prepared and screened. There is no mention of a high throughput format.

The residue-substituted polypeptides are then tested for activity with a particular target and the activity compared to the parent polypeptide to identify the amino acid residues in the active domain that are involved in the interaction with the target. Further, once the active amino acids in the active domain are identified they can be further modified to alter the interaction of the parent protein with one or more target. But, Wells *et al.* **does not teach or suggest replacing each of the amino acid residues identified as involved in the interaction with the target with every other amino acid** and further screened.

Wells *et al.* does not teach or suggest a high throughput method of directed evolution. Wells *et al.* studies proteins individually in order to indentify active domains of a protein. Wells does not teach or suggest replacing amino acids along the full-length of a protein and testing each one-by-one to identify hits. The methods of Wells *et al.* is exemplified by substitution and modification of human growth hormone to alter its interaction with its somatogenic receptor in order to assess the structure/function relationship. In determining the active domain amino acid residues, three active domains were identified based on segment-substituted polypeptides. Residues in the three active domains were then replaced sequentially with alanine, yielding a total of 63 mutants. Thus, only part 2 of Wells' *et al.* 3-part method is similar to part 1 of the instantly claimed method, and its similarity is only to the extent that Wells *et al.* replaces amino acids with a scanning amino acid. The similarity ends there, since Well *et al.* only scans the identified domain, not the full-length of the protein as required by the instant claims.

Residues that alter the interaction with the somatogenic receptor were further modified in the human growth hormone polypeptide, **but not** by replacing each with every other amino acid and testing each for activity. Wells *et al.* specifically states (*i.e.,* column 17, lines 29 *et seq.*) that once active amino acids are identified, isoteric amino acids may be substituted. Isoteric substitutions (listed in Table II of Wells *et al.*) are used to minimize effects on conformation. Wells *et al.* **does not** teach or suggest replacing amino acids that affect activity with **all** other amino acids. Thus, Wells *et al.*, **does not teach the further steps (corresponding to steps e-g of claim 1, steps d-f of claim 27) of** then replacing the amino acids **at each of the hit loci, one-by-one, with every other amino acid** to identify amino acids that confer a desired change in activity or property the protein. Wells *et al.*, identifies and studies active domains. Wells *et al.* does not teach or suggest a method in which the full length of protein is assessed by changing each residue, one-by-one, and then, replacing the amino acids at each and every hit with all amino acids.

Further, Wells is not properly combinable with Pederson *et al.*, since the methods in each bear no relationship to the other. Even assuming motivation to combine these references post-KSR, the methods in each cannot be combined in any meaningful way. Wells is a method or identifying active domains, and prepares proteins with replaced domains to assess the effects, identifies domains of interest and modifies amino acids in the domains. This method has nothing in common with the method of Pederson *et al.*, which describes a method for separating individual clones into wells. Such method has nothing related to the method of Wells *et al.* so it cannot be combined with the method of Wells *et al.*

Pederson *et al.*

Pederson *et al.*, does not cure the deficiencies of Wells *et al.*, nor does it in combination with Wells *et al.* result in the instantly claimed methods. As discussed above, Wells *et al.* fails to teach or suggest numerous elements of the instant claims, including, but are not limited to, 1) a high throughput method; 2) replacing residues along the full the length of the protein one-by-one, and 3) steps e-g and steps d-r of claims 1 and 27, respectively, in which hit positions are replaced with every other amino acid and separately tested for activity to identify proteins that have a predetermined activity/property, and 4) a method of directed evolution..

Pederson *et al.* teaches a screening method that uses a spatial array to separate molecules in each position of the array for screening. The identity of the molecule in each position in a spatial array is not known **but must be** determined by various screening assays. Pederson *et al*, teaches that by using this method, variation in expression level still exist, but that uniform growth of identical clones in different wells is achieved. In this instance then, the spatial array is not being used to individually introduce and express mutant proteins, but instead is being used to individually grow cells and express the mutant proteins. The identity of each clone in the array is not known *a priori* and each clone must be further characterized, such as by sequencing to identify the encoded protein.

Hence, Pederson does not teach or suggest using an addressable array for the individual introduction and expression of mutant proteins. The teachings of Pederson point to the fact that the protein molecules are not individually expressed in host cells in the array. Rather, Pederson teaches, such as in Example 5 (see e.g., at page 54, line 14), that spores already expressing **a mixture** of mutants from a mutant library are introduced into a spatial array for screening. For example, distinct mutant **pools** are added to each array so that one spore in average is inoculated per well meaning that some wells will contain no spores and some wells will contain more than one spore. **Thus single clones are arrayed, but they are picked from mixtures.** As a result, one does not *a priori* know the identity of a spore or spores in a particular locus in the array (hence it is not "addressable" as required in the instant claims). Example 9 describes another strategy ,which also does not teach or suggest that mutants are individually introduced into host cells, as required by the instant claims. Example 9 (see e.g., page 60, beginning at line 26), describes the generation of a mutant library of lipase, which library is transformed into fungal protoplasts as a mixture using alginate balls. Hence, mixtures of mutants are produced.

Also, in the methods of Pederson, the mutants expressed in each array are not the mutants that have been generated one-by-one such that each variant nucleic acid encodes a mutant protein that differs by one replacement amino acid from the original nucleic acid molecule. For example, Example 5 (see e.g., page 54, beginning at line 29) teaches the generation of mutant Shearzyme®. As taught in Pederson, random mutants of Shearzyme® are generated by UV-irradiation. Thus, the identity and number of mutations in each mutant protein is not known without further sequencing. Another example, Example 9 teaches an error-prone PCR-based mutation of lipase, which also results in non-targeted, randomized

mutation of lipase to generate a **library** of mutants. Thus, Pederson *et al.* does not teach or suggest any elements of the instantly claimed method and does not cure the deficiencies in Wells *et al.* nor does its combination with Wells *et al.* result in the instantly claimed methods.

Analysis

The combination of teachings of the cited references fails to result in the method of any pending claim.

Neither Pederson *et al.*, nor Wells *et al.*, singly nor in any combination, teaches numerous elements of the instantly claimed methods. As discussed above Wells *et al.* fails to teach steps in the instantly claimed method, including: (1) a method of directed evolution; (2) a high throughput method; (3) introducing mutations into a protein one-by-one **along the full-length** of the protein and individually expressing each protein; (4) identifying the modified protein that has altered activity/property of interest (a HIT) by virtue of its locus in an addressable array; and (5) individually replacing the hit amino acids with all other amino acids and individually expressing and screening the proteins with modified hits to identify LEADs that have the predetermined activity/property. As discussed, Wells provides a method for identifying active domains and studying protein structure function relationship; its method requires substitution of a putative domain with a domain from an analogous protein and assessing its effect. Once a domain is identified, amino acids in the domain are modified to assess their effects. Wells *et al.*, however, provides not teachings or suggestions any of (1)-(5). Pederson *et al.* similarly fails to teach any of these elements.

Therefore, the combination of teachings of these references does not and cannot result in the instantly claimed method. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness.

In addition, each reference is directed to a method that is separate and distinct from the other and not amenable to modification. Wells *et al.* provides a method for studying protein structure/function. Pederson *et al.* is directed to a method involving clonal arrays, in which clones are picked from mixtures and arrayed separately. In the methods described by Pederson, mixtures of clones are produced. Wells *et al.*, does not produce mixtures of clones; thus, the method of Pederson *et al.* cannot be combined with Wells *et al.* .

Therefore, the combination of Wells *et al.* and Pederson *et al.* does not result in the instantly claimed methods. The combination is deficient in failing to teach at least elements

(1)-(5) discussed above. In addition, the methods of Wells *et al.* and Pederson *et al.* cannot be combined. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness.

Rebuttal to the comments of the Examiner

1. The Examiner states that:

> Wells *et al.* teaches a method of producing a plurality of separate sets of nucleic acid molecules that encode modified forms of a target protein (page 6, paragraph 45-65), where a residue substituted DNA sequence encodes a residue substituted polypeptide where each polypeptide contains a single substitution at a different amino acid within each segment of a protein (Wells describes proteins as being comprised of segments) . . .

Wells does not describe proteins as being comprised of segments. As discussed above, Wells *et al.* produces segmented proteins by replacing segments of one protein with a corresponding segment of another protein that exhibits a desired activity. The resulting protein is a segmented protein, it contains a plurality of different amino acids from the parent protein. The purpose is to identify active domains in a particular protein. The segmented proteins are tested for a particular activity (i.e. receptor binding) to see whether the segment confers a different activity (*i.e.*, receptor binding activity) on the protein. If it does, then Wells *et al.* concludes that that particular replaced segment is involved in the particular activity. Wells *et al.* then replaces each amino acid in the segment in the original protein to identify particular amino acids that affect the activity in the domain. Wells *et al.* does **not** replace every amino acid along the full-length of the protein **nor can it** provide a suggestion to do so since the focus of its method is on first identifying domains that are involved in an activity, and then replacing amino acids in the domain.

2. The Examiner continues:

> . . . screening the encoded proteins for a predetermined property that differs from the target protein by at least a 100% change (i.e. two-fold increase or decrease in Kd as compared to the parent protein) (column 17, lines 29-43); identifying each mutated protein as a hit and a designated hit position (i.e. active amino acid residues) (column 17, lines 29-43).

The reference section in column 17, does not describe a step of screening a protein for a predetermined activity in a method of protein evolution. The change in Kd to which the Examiner refers is the cut-off for concluding that a particular residue is active in interaction with a particular target. See, lines 38-43, column 17, which state:

> In general, a two-fold increase or decrease in KD indicates that the residue substituted is active in the interaction with the target, a two-fold increase or decrease in kcat/Km relative to the parent enzyme indicates that an active residue has been substituted.

Thus, the referenced lines describe when a residue in a protein is deemed to be one that affects a particular activity. It is not teaching that the protein identified has a predetermined activity. Wells *et al.* is screening to identify the activity of a residue. In the instant method, the activity of a protein is screened.

3. The Examiner also says that:

> Applicants also state that Wells *et al.* do not teach the further step of replacing the amino acids of each hit loci to identify amino acids that confer a desired change in activity. However, Wells *et al.* teach that once the amino acid position is identified, other amino acids may be substituted into that position (column 15, line 65-column 17, line 60).

As discussed above, Well *et al.* states that the amino acids identified as important for an interaction are replaced with isoteric amino acids, not every amino acid. Hence, Wells *et al.* does not teach steps e-g of claim 1 or d-f of claim 27.

4.

> Applicants have responded to the Pedersen *et al.* by stating that the clones used in the array are not known or identified. Because the clones are not known, the array is not addressable. However, the Examiner was not relying on Pedersen *et al.* to teach known clone cells, rather the Examiner was relying on Pedersen *et al.* to teach the used of a spatial array that may be used as an addressable array when combined with Wells *et al.* Wells et a. do teach individually introducing nucleic acids into a host cell (column 13, lines 36-53). Pedersen *et al.* demonstrates how these host cells may be grown in an addressable array.

Pederson *et al.* does not teach an array that can be addressable when combined with Wells *et al.* Pederson teaches an array in which individual clones from mixtures of clones. are placed into a well so that a single clone grows in each well. Wells *et al.* does not produce mixtures of clones. Therefore the method of Pederson *et al.* is not applicable to the method of Wells *et al.*

Furthermore, as discussed above, even if Pederson *et al.* is relied upon to teach that clones can be placed in microwell plates, placing the clones of Wells *et al.* in microwell plates does not result in the instantly claimed methods. The combination of Wells *et al.* and Pederson *et al.* still fails to teach or suggest elements of all pending claims that include: (1) a method of directed evolution; (2) a high throughput method; (3) introducing mutations into a protein one-by-one along the full-length of the protein and individually expressing each protein; and (5) individually replacing the hit amino acids with all other amino acids and

individually expressing and screening the proteins with modified hits to identify LEADs that have the predetermined activity/property

Remaining claims

The remaining claims are rejected over combinations of Wells *et al.* and Pederson *et al.* in view of further references alleged to teach elements of dependent claims, not the independent claims, these rejections fail since dependent claims necessarily include all limitations of the independent base claims, Wells *et al.* and Pederson *et al.*, fail to teach or suggest the methods of the independent claims.

Claims 24, 28 and 29

Claims 24, 28 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wells *et al.* (US 6,013,478) in view of Pedersen *et al.* (W001/32844) as applied to claims 1-3, 5-10, 14, 15, 17-23, 27, 43 and 44 above, and further in view of Berlioz *et al.* (US 5,925, 565) because Berlioz *et al.* teaches "assessing the titer of the viral vectors after transfection for each set of eukaryotic cells (column 14, lines 39-65) and where the viral vector encodes for a protein involved in viral replication (column 5, lines 35-65)." The Examiner concludes that it:

> would have been obvious to one of ordinary skill in the art at the time of the invention to modify the methods taught by Wells *et al.* and Pedersen *et al.* with Berlioz *et al.* in order to study the effects of the protein in an eukaryotic setting. Berlioz *et al.* teaches a method that allows eukaryotic cells, such as a human cell, to express a desired protein (column 6, lines 5-22) for the purpose of producing a therapeutic treatment (column 7, lines 15-25). Wells *et al.* and Pedersen *et al.* methods teach screening for different proteins that exhibit a desired biological, chemical, or physical property. Thus one of ordinary skill in the art seeking to create a new therapeutic treatment, would be motivated to use Wells *et al.* and Pedersen *et al.* methods to design a product and use Berlioz *et al.*'s method to express the protein in an eukaryotic cell.

This rejection respectfully is traversed.

The rejected claims

Claim 24 includes the method of claim 1 and recites that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b).

Claim 28 recites that the at step (f) in the method of claim 27 the titer of the viral vectors in each set of cells is determined; and claim 29 recites that in the method of claim 28, the target protein is a protein involved in viral replication..

Analysis

As discussed above, the combination of teachings of Wells *et al.*, and Pederson *et al.*, fails to teach or suggest teach any or all limitations of independent claims 1 and 27 or any of the pending claims, and hence fails to teach or suggest any or all elements of the rejected dependent claims. Berlioz *et al.* fails to cure the deficiencies in the teachings of these references since Berlioz *et al.* does not teach or suggest a high throughput directed evolution method that includes producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, where all nucleic acid molecules in a set encode the same modified protein and the proteins are modified along their full-length or the full-length of a domain (step (a)); arraying cells containing the nucleic acid molecules such that the identity of the encoded protein is known *a priori*(step (b)), and/or individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)), nor any other steps or elements of the methods as claimed.. Therefore the combination of teachings of Wells *et al.*, Pederson *et al.*, and Berlioz *et al.* does not result in the methods of claims 7, 24 and 27-29.

The rejection of claims 25, 26, 32 and 33

Claims 25, 26, 32, and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wells *et al.* in view of Pedersen *et al.* in view of Berlioz *et al.* as applied to claims 1-3, 5-10, 14, 15, 17-23, 27, 32, 33, 43 and 44 above, and further in view of Drittanti *et al.* (Gene Therapy (2000) Volume 7, pages 924-929) because Drittanti *et al.* teaches real time virus titering (page 925); using tagged replication and expression enhancement (page 926, right column); and where the process is automated and computer controlled (page 925, left column under Figure 1), which are elements of claims 25, 26, 32 and 33. The Examiner concludes that it would:.

> have been obvious for one of ordinary skill in the art at the time of invention to modify the methods of Wells *et al.*, Pedersen *et al.*, and Berlioz *et al.* with Drittanti *et al.* to gain the benefit of determining the effectiveness of viral vectors. Berlioz *et al.* teach that one of his goals is to create an effective and stable viral vector (column 1, lines 10-17). Part of their method requires that they assess the titer of the viral vectors after transmission. Drittanti *et al.*'s method provides further insight into the stability and efficacy of the vector by offering real time titering. Thus one of ordinary skill in the art would be motivated to combine the methods of Wells *et al.* Pedersen *et al.*, and Berlioz *et al.* with Drittanti *et al.* in order to gain the benefit of assessing the stability and efficacy of viral vectors.

This rejection respectfully is traversed.

Without conceding whether Drittanti *et al.*, which is authored by inventors of the

instant application, is effective as prior art against any claims in this application, it does teach real time tittering. Drittanti *et al.*, however, does not teach any elements in the independent claims missing from the combination of teachings of Wells *et al.* in view of Pedersen *et al.* in view of Berlioz *et al.* Therefore, the combination of teachings of Drittanti *et al.* with those of Wells *et al.* in view of Pedersen *et al.* in view of Berlioz *et al.* does not result in the methods of any of claims 25, 26, 32 and 33.

The rejection of claims 30 and 31

Claims 30 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wells *et al.* and Pedersen *et al.*, as applied to claims 1-3, 5-10, 14, 15, 17-23, 27, 43 and 44 above, and further in view of Persson *et al.* (Journal of Virology (1985) Volume 54, pages 92-97) because Persson *et al.* is alleged to teach a method that uses a Hill analysis for determining the rate in which host cells are infected with viruses (abstract, page 94, left column). The Examiner concludes that:

> [i]t would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Wells *et al.* and Pedersen *et al.* with the method of Persson *et al.* to gain the benefit of determining if the plasmids or vectors are infecting the host cells. Wells *et al.* teach creating host cells with desired nucleic acids. In such a method, it would be desirable to determine the rate of infection in order to determine how to structure an experiment (e.g., incubation times, concentration, etc.). Persson *et al.* provide a method of determining the rate of infection. Thus one of ordinary skill in the art would be motivated to combine the methods of Wells *et al.* and Pedersen *et al.* with the method of Persson *et al.* to gain the benefit of determining the rate of infection of host cells to structure his experiments..

This rejection respectfully is traversed.

Claim 31, which is dependent on claim 30, which includes the elements a)-g) as discussed above, and further includes recites that the "performance of the screened proteins is evaluated by a Hill analysis. Persson *et al.* does not teach or suggest a Hill analysis as claimed, nor does Persson *et al.* teach or suggest the elements of claim 30, missing from the combination of teachings of Wells *et al.*, and Pederson *et al.* Therefore, the combination of teachings Wells *et al.*, Pederson *et al.* and Persson *et al.* does not result in the methods of claims 30 and 31. Therefore for these reasons and those discussed above, the Examiner has failed to set forth a *prima facie* case of obviousness of any of the pending claims.

* * *

In view of the above, reconsideration and allowance respectfully are requested.

Respectfully submitted,

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